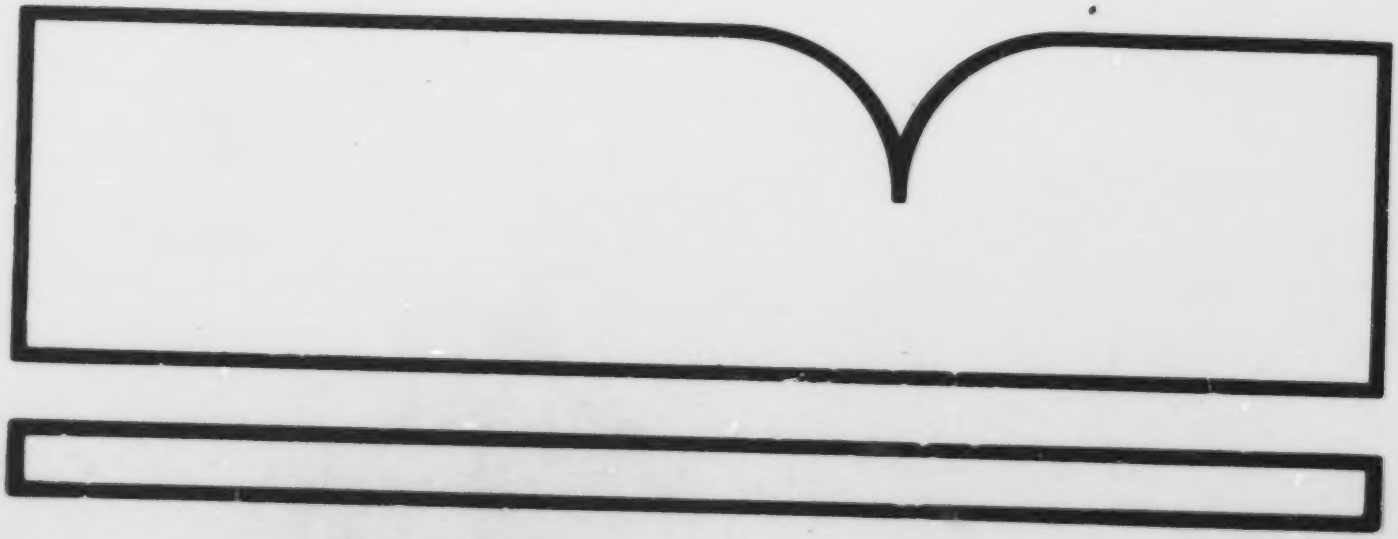
PB90-245275

Assessing Detoxification and Degradation of Wood Preserving and Petroleum Wastes in Contaminated Soil

(U.S.) Robert S. Kerr Environmental Research Lab., Ada, OK

1990



U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before complet

1. REPORT NO. EPA/600/J-90/099	2.
3. PB90-245275	
4. TITLE AND SUBTITLE ASSESSING DETOXIFICATION AND DEGRADATION OF WOOD PRESERVING AND PETROLEUM WASTES IN CONTAMINATED SOIL	5. REPORT DATE
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Wayne Aprill, Ronald C. Sims, Judith L. Sims,[1] and John E. Matthews[2]	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS 1. Utah State University Logan, UT 84322-4110 2. Robert S. Kerr Environmental Research Laboratory P.O. Box 1198 Ada, OK 74820	10. PROGRAM ELEMENT NO. CBWD1A 11. CONTRACT/GRANT NO. CR-813211
12. SPONSORING AGENCY NAME AND ADDRESS Robert S. Kerr Environmental Research Laboratory - Ada U.S. Environmental Protection Agency P.O. Box 1198 Ada, OK 74820	13. TYPE OF REPORT AND PERIOD COVERED Journal Article 14. SPONSORING AGENCY CODE EPA/600/15
15. SUPPLEMENTARY NOTES Published: WASTE MANAGEMENT & RESEARCH, Vol. 8, No. 1, pp. 45-65, February 1990.	

16. ABSTRACT

This study was undertaken to evaluate in-situ soil bioremediation processes, including degradation and detoxification, for wood preserving and petroleum refining wastes at high concentrations in an unacclimated soil. The soil solid phase, water soluble fractions of soil, and column leachates were evaluated. A mutagenic potential assay (Ames assay) and an aqueous toxicity assay (Microtox™ assay) were used to evaluate detoxification; high performance liquid chromatography was used to evaluate chemical concentration and degradation for eight polynuclear aromatic hydrocarbons (PAHs). The group of noncarcinogenic PAHs studied demonstrated greater degradation, ranging from 54-90% of mass added for the wastes; the carcinogenic group of PAHs studied exhibited degradation ranging from 24-53% of mass added. Although no mutagenicity was observed in waste/soil mixtures after one year, Microtox™ toxicity was observed in water soluble fractions and in leachate samples. Integration of information concerning degradation of hazardous constituents with bioassay information represents an approach for designing treatability studies and for evaluating effectiveness of in-situ soil bioremediation. When combined with information from waste, site, and soil characterization studies, data generated in treatability studies may be used in predictive models to: (1) evaluate effectiveness of on-site soil bioremediation; (2) develop appropriate containment structures to prevent unacceptable waste transport from the treatment zone; and (3) design performance monitoring strategies.

→ con't on pg 45

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field, Group

18. DISTRIBUTION STATEMENT RELEASE TO THE PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 22
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE A03

EPA Form 2220-1 (Rev. 4-77) PR

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA 22161

EPA/600/J-90/099

Waste Management & Research (1990) **8**, 45–65

PB90-245275

ASSESSING DETOXIFICATION AND DEGRADATION OF WOOD PRESERVING AND PETROLEUM WASTES IN CONTAMINATED SOIL

Wayne Aprill*, Ronald C. Sims*†, Judith L. Sims*, and John E. Matthews‡

(*Received March 1989, revised September 1989*)

This study was undertaken to evaluate in-situ soil bioremediation processes, including degradation and detoxification, for two types of wood preserving wastes and two types of petroleum refining wastes at high concentrations in an unacclimated soil. The soil solid phase, water soluble fractions of the soil, and column leachates were evaluated. Two bioassays, a mutagenic potential asay (Ames assay) and an aqueous toxicity assay (Microtox™ assay) were used to evaluate detoxification; high performance liquid chromatography was used to evaluate chemical concentration and degradation for eight polynuclear aromatic hydrocarbons (PAHs). The group of non-carcinogenic PAHs studied demonstrated greater degradation, ranging from 54–90% of mass added for the four wastes; the carcinogenic group of PAHs studied exhibited degradation ranging from 24–53% of mass added. Although no mutagenicity was observed in waste/soil mixtures after one year of treatment, Microtox™ toxicity was observed in water soluble fractions and in leachate samples. An integration of information concerning degradation of hazardous constituents with bioassay information represents an approach for designing treatability studies and for evaluating the effectiveness of in-situ bioremediation of contaminated soil/waste systems. When combined with information from waste, site and soil characterization studies, the data generated in treatability studies may be used in predictive mathematical models to: (1) evaluate the effectiveness of use of on-site bioremediation for treatment of wastes in soil systems; (2) develop appropriate containment structures to prevent unacceptable waste transport from the treatment zone; and (3) design performance monitoring strategies.

Key Words—Hazardous wastes, bioremediation, in-situ treatment, contaminated soils, polynuclear aromatic hydrocarbons, bioassays, degradation, detoxification, wood preserving wastes, petroleum refining wastes.

1. Introduction

In-situ biological remediation of soils contaminated with wastes containing polynuclear aromatic hydrocarbons (PAHs) is a treatment technology that is potentially technically feasible and meets the goal of achieving permanent remedies for clean-up, as encouraged by the U.S. Environmental Protection Agency (USEPA) for implementation of The Superfund Amendments and Reauthorization Act (SARA) of 1986. The use of naturally occurring micro-organisms to accomplish degradation and detoxification of hazardous constituents for the protection of health and the environment is consistent with the

*Utah State University, Department of Civil and Environmental Engineering, Logan, Utah 84322-4110, U.S.A.

†Address correspondence to R. C. Sims.

‡Robert S. Kerr Environmental Research Laboratory, U.S. Environmental Protection Agency, Ada, Oklahoma 74820, U.S.A.

0734-242X/90/010045 + 21 $03.00/0

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.

©1990 ISWA

philosophical thrust of SARA and is the goal of on-site bioremediation of hazardous waste contaminated soils. The subject of bioremediation of contaminated soils, including applications and limitations of the technology, has been addressed at several recent scientific meetings and conferences (Omenn 1987, Engineering Foundation 1988, HMCRI 1988, AWMA/EPA 1989, and USEPA 1989). With regard to wood preserving contaminated sites, McGinnis *et al.* (1988) have stated that reliable, safe, economical bioremediation techniques using soil systems are attractive and warrant thorough study and evaluation.

Prior to implementation of an in-situ biological remediation technology for a soil contaminated with a complex waste, an evaluation of the potential of the contaminated soil system to accomplish detoxification and degradation of hazardous constituents present in the waste should be conducted. Treatability studies can be used to evaluate detoxification and degradation processes as they affect the fate and behavior of hazardous constituents in a contaminated soil. Treatability studies can provide specific information that can be used to: (1) determine migration (leaching) potential; (2) correlate chemical disappearance with changes in bioassay response; (3) compare treatment of different wastes under similar experimental conditions; and (4) evaluate approaches for enhancing treatment. Soil that has been previously exposed to waste and has been acclimated, or unacclimated soil that has not been previously exposed to a waste, can be used. Persistent hazardous constituents that occur as co-substrates in a matrix where different hydrocarbons are present that serve as readily available growth substrates may be degraded through the process of co-oxidation (Foster 1962*a*,*b*). Apparent degradation refers to measurement of changes in concentrations of specific constituents in solvent extracts of soil samples with time of incubation. Results of treatability studies are often reported in terms of apparent degradation of hazardous constituents.

PAH compounds are a group of hazardous organic substances of critical public health and environmental concern due to the following characteristics: (1) chronic health effects (e.g. carcinogenicity); (2) microbial recalcitrance; (3) high bioaccumulation potential; and (4) low removal efficiency in traditional waste treatment processes (Herbes & Schwall 1978). Though PAH compounds are considered recalcitrant, microbial metabolism has been demonstrated in pure microbial cultures and in soil systems (Gibson *et al.* 1975, McKenna 1977, Cripps & Watkinson 1978, Cerniglia *et al.* 1983, Bossert *et al.* 1984, Cerneglia & Yang 1984, Coover & Sims 1987, Park 1988, and Park *et al.* 1988).

Petroleum refining and wood preserving wastes are two categories of complex industrial wastes that contain PAH compounds identified as hazardous constituents in 40 CFR Part 261, Appendix VIII, and are of concern to the USEPA due to volume and toxicity characteristics (USEPA 1986*c*, and McGinnis *et al.* 1988). Wastes from the petroleum industry have been shown to contain PAH compounds (USEPA 1986*b*) and to exhibit mutagenic activity (Donnelly *et al.* 1985). Creosote sludge and a mixture of creosote and pentachlorophenol (PCP) sludge are two common types of wood preserving wastes. Creosote contains many constituents that have been reported to be mutagenic, teratogenic, fetotoxic and/or toxic (USEPA 1974, 1984). Bos *et al.* (1983, 1984) determined that the mutagenicity of creosote was probably due to the presence of mutagenic polycyclic aromatic hydrocarbons (PAHs). PCP, when used as a wood preservative, was usually mixed with petroleum products or added to creosote. PCP is also of environmental concern because it has been reported to be toxic to humans and to aquatic life (USEPA 1986*a*). The level of impurities in PCP may also be important, for

most technical PCP samples contain the higher-chlorinated dibenzodioxins and dibenzofurans. The dioxin usually present in the highest concentration is the comparatively non-toxic octachlorodibenzo-p-dioxin (OCDD). The highly toxic tetrachlorodibenz-p-dioxin (TCDD) is usually not present, but the toxic hexachlorodibenzo-p-dioxin (HCDD) and heptachlorodibenzo-p-dioxin (HpCDD) isomers are usually present (Crosby 1981).

There have been few published studies in which bioassays have been combined with chemical assays to evaluate the extent of both detoxification and degradation of hazardous substances in soil systems and to characterize the toxicity of potential leachates. Chemical analyses may be used to define the types and concentrations of hazardous compounds in a soil, but the results must be extrapolated to estimate the toxicological effects on biological systems (Donnelly *et al.* 1986).

Two bioassays that have been used to evaluate wastes in soil systems are the Ames *Salmonella typhimurium* mammalian microsome assay and the Microtox™ test system. The Ames assay is a measure of the mutagenic potential of hazardous compounds (Ames *et al.* 1975, Maron & Ames 1983) and has been widely used to evaluate environmental samples (Sims *et al.* 1984, 1985, 1986, 1987, and 1988). A high correlation has been shown between carcinogenicity and mutagenicity, where about 90% of known carcinogens tested mutagenic in the Ames assay (McCann *et al.* 1975). Special strains of *Salmonella typhimurium* that require histidine to grow are used to test for mutagenicity. When plated on a histidine-free medium, the only bacteria able to form colonies are those that have reverted to the "wild" state and are able to produce their own histidine. Without the addition of test chemicals, this back mutation occurs at a rate specific to each strain type (spontaneous reversion rate). The addition of chemicals that are mutagenic increases the reversion rate. Several dose levels of a chemical, mixture of chemicals, or an environmental sample are added to obtain a dose response. Some mutagens act directly on the bacterial cells while others require activation by mammalian microsomes. These microsomes are generally obtained from liver extracts of Aroclor 1254-induced rats (i.e. rats injected with the polychlorinated biphenyl (PCB), Aroclor 1254). The extract, referred to as the S-9 fraction, contains enzymes that metabolically convert certain chemicals to active mutagens, simulating the activity that occurs in living mammalian systems. Several strains of *Salmonella typhimurium* have been developed in order to detect different types of mutagens. The recommended strains for general mutagenicity testing include TA97, TA98, TA100, TA102. TA97 and TA98 detect frameshift mutagens. TA100 detects mutagens causing base-pair substitutions, while TA102 detects a variety of mutagens not detected by the other strains.

The Microtox™ assay is an aqueous general toxicity assay that measures the reduction in light output produced by a suspension of marine luminescent bacteria in response to an environmental sample (Matthews & Hastings 1987). Bioluminescence of the test organism depends on a complex chain of biochemical reactions. Chemical inhibition of any of the biochemical reactions causes a reduction in bacterial luminescence. Therefore the Microtox™ test considers the physiological effect of a toxicant and not just mortality. Matthews and Bulich (1984) have described a method of using the Microtox™ assay to predict the land treatability of hazardous organic wastes. Matthews and Hastings (1987) described a method using the Microtox™ assay to determine an appropriate range of waste application loading for soil-based treatment systems. Symons and Sims (1988) utilized the assay to assess the detoxification of a complex petroleum waste in a soil environment. The assay was also included as a recommended bioassay in the USEPA Permit Guidance Manual on Hazardous Waste Land Treatment Demonstrations (USEPA 1986*b*).

This study was undertaken to evaluate in-situ soil bioremediation processes, including degradation and detoxification, for two types of wood preserving wastes and two types of petroleum refining wastes at high concentrations in an unacclimated soil. Bioassays and chemical analyses were used to quantify detoxification and degradation in the soil solid phase, in water soluble fractions of the soil, and in leachates from the soil system. Two bioassays, a mutagenic potential assay (Ames assay) and an aqueous toxicity assay (Microtox™ assay) were used to evaluate detoxification of soils systems contaminated with the wastes. Degradation of individual PAH compounds in the soils was monitored using high performance liquid chromatographic (HPLC) analyses of soil extracts.

2. Materials and methods

2.1 Selection of materials

Four complex wastes were selected for use in this study. Two wastes were byproducts of the petroleum refining industry, American Petroleum Institute (API) separator sludge and slop oil emulsion solids. Creosote sludge and PCP-creosote mixed sludge were used from the wood preserving industry. Physical and chemical characteristics of the wastes are presented in Table 1.

TABLE 1
Physical and chemical characteristics of complex wastes (USEPA 1986*c*)

Complex waste	Water content (%)	Total residue (103°C) (mg g^{-1})	Total volatile suspended residue (550°C) (mg g^{-1})	Oil and grease content (%)	pH (units)
API separator sludge	47* ± 2.8	257* ± 32	33.2* ± 10.0	35* ± 2.5	4.9
Slop oil emulsion solids	0.1 ± 0	227 ± 27	1.77 ± 0.19	46 ± 4.9	3.9
Creosote sludge	33 ± 0.7	522 ± 9	229 ± 36	37 ± 1.2	5.5
PCP-creosote mixed sludge	28 ± 0.7	422 ± 19	189 ± 10	52 ± 1.5	5.4

* Mean of three replicates ± standard deviation.

The soil used as a treatment medium was collected from the top 15 cm of a Kidman sandy loam soil (Typic Haplustoll, Utah). Selected physical and chemical characteristics of the soil are shown in Table 2. The soil was prepared by air-drying and sieving through a 2 mm sieve prior to use. The soil had not been previously exposed to the wastes, and therefore was unacclimated.

TABLE 2
Soil physical and chemical characteristics of the upper 15 cm of Kidman sandy loam soil (USEPA 1986*c*)

Characteristic	Value
Physical	
Texture	Sandy loam
Moisture retention at	
0 bar	24%
-1/3 bar	20%
−15 bar	7%
Bulk density	1.49 g cm^{-3}
Chemical	
pH	7.9
Cation exchange capacity (CEC)	10.1 meq $100\,g^{-1}$
Organic carbon	0.5%
Electrical conductivity (EC) of saturated extract	0.2 mmhos cm^{-1}

2.2 Experimental design

An experimental approach using batch reactors and soil columns was implemented in the laboratory to evaluate the degradation, detoxification, and mobility of each complex waste in the unacclimated sandy loam soil.

2.2.1 Batch reactor incubation study

Batch reactors were prepared consisting of 200 g of soil in 600 ml glass beakers. One waste was mixed into the soil in each reactor on a percent waste wet weight/soil dry weight basis (Table 3). Applied waste concentrations were determined using two short-term bioassays (Microtox™ toxicity assay and soil respiration assay) to assess toxicity of the waste to microbial activity (USEPA 1986*a*). Concentrations of each waste were chosen that were toxic in the Microtox™ assay, but were not toxic in a soil respiration assay that was based upon a reduction of carbon dioxide evolution for determination of toxicity. Two batch reactors were prepared for each soil/waste loading rate. Two batch reactors containing soil with no waste applied were prepared as controls. The soil in each reactor was wetted to approximately −1/3 bar matric potential (a measure of the energy required to extract water from soil pores to overcome capillary and adsorptive forces)

TABLE 3
Soil loading rates for complex wastes (USEPA 1986*c*)

Complex waste	Loading rate	
	Percentage waste (wet weight/soil dry weight)	Percentage oil and grease
API separator sludge	12.0	4.2
Slop oil emulsion solids	12.0	5.5
Creosote sludge	1.0	0.4
PCP-creosote mixed sludge	0.3	0.16

with distilled, deionized water (DDW). Contents of one batch reactor for each soil/waste loading rate and one control reactor were extracted for determination of initial PAH concentrations and mutagenic potential, using the procedure for organic extractions outlined in Section 2.3.1. The remainder of the batch reactors were covered with a polyethylene film (GladWrap, Dow Chemical Company), which allows the passage of oxygen, but retains moisture (Bossert 1984, and Coover *et al.* 1987). The reactors were incubated for 354 days in a $20 \pm 2°C$ constant temperature room in the dark to prevent photodegradation of the PAH compounds. The waste/soil mixtures in the reactors were mixed weekly to maintain aerobic conditions. Moisture levels were maintained between -3 and $-1/3$ bars. At the end of the study, the soil/waste mixtures were extracted for determination of final PAH concentrations and mutagenic potential according to the procedures given in Section 2.3.1.

2.2.2 Soil column studies

Column studies were conducted to generate samples for evaluation of potential toxicity of leachates from the contaminated soil systems, as determined by the Microtox™ toxicity test. Studies were conducted at the beginning and end of the incubation study. For the initial column study, duplicate columns with wastes incorporated in the surface of the soils were prepared for each waste/soil combination. Control columns with no waste added were also prepared. Mobility of waste constituents in the columns was evaluated under saturated conditions. Glass columns (50 cm × 5 cm) fitted on the bottom with glass frits and Teflon™ stopcocks were packed with 35 cm (912 g) of air-dried unacclimated Kidman sandy loam soil. One hundred and ninety-five grams of waste were incorporated in the top 8 cm of the soil. Initially the columns were gravimetrically back-fed with DDW in order to replace air from pore spaces with water. During the period of study, water was added to the soil by the continuous maintenance of an 8 cm head of water at the top of each column. Leachates were collected at the bottom of the columns. The flow rate was constant at one pore volume (approximately 300 ml) per day. The study was conducted until 15 pore volumes were collected. Leachates were analysed directly for Microtox toxicity at 1,3,5,7,9,11,13, and 15 pore volumes following standard Microtox procedures™, as referenced in Section 2.4.

For the second column study, waste-amended soils collected from the soil batch reactors that had been incubating for approximately one year, were incorporated into the surface soil of columns containing Kidman sandy loam. Column preparation and operation were identical to that of the initial study, but with 49 g of waste-amended soils incorporated into the top of the columns.

2.3 Extraction procedures for preparation of samples for chemical and toxicological analyses

2.3.1 Sample preparation for analysis of PAH constituents and measurement of mutagenic potential

For each waste, samples for analysis of extractable PAH constituents and mutagenic potential were prepared by placing a 10 ml aliquot of a waste in a 225 ml centrifuge tube and diluting with 60 ml of DDW. The diluted sample was homogenized with a Tekmar Tissumizer® (Tekmar Co., Cincinnati, OH) blending probe for 30 s. The solution was then adjusted to pH 11 with 6N sodium hydroxide. Eighty millilitres of dichloromethane were then added to the slurry, homogenized for 30 s with the Tissumizer, and centrifuged

for 30 min at 2500 r.p.m. The dichloromethane phase was saved. The procedure was repeated two more times. The three dichloromethane extracts were combined, passed through an anhydrous sodium sulfate drying column, and concentrated to approximately 5 ml in a Kuderna-Danish (K-D) apparatus. This concentrate contained the extractable basic and pH neutral organic constituents of the waste. To extract the acidic constituents of the PCP-creosote mixed sludge, the waste-DDW mixture was adjusted to pH 1 with 6N hydrochloric acid, and the extraction, drying, and concentration steps were repeated.

For each soil/waste mixture, preparation of samples for analysis of PAH constituents and mutagenic potential followed the method described by Coover *et al.* (1987). A 10 g subsample of soil/waste mixture (at $-1/3$ bar moisture) from a batch reactor was extracted with 250 ml of the solvent dichloromethane with the Tissumizer® blending probe. The solvent extract was decanted from the mixture, passed through an anhydrous sodium sulfate drying column, and concentrated to approximately 1 ml in a KD apparatus.

2.3.2 Sample preparation for measurement of Microtox™ toxicity

Preparation of samples for determination of the water soluble fraction (WSF) toxicity of waste and soil/waste mixtures, as determined by the Microtox™ toxicity assay, was accomplished using a procedure similar to the USEPA procedure for obtaining extracts for use in the Extraction Procedure (EP) toxicity test (USEPA 1982). A 100 g sample of a soil/waste mixture and 400 ml of DDW were added to a glass extraction vessel. The extraction vessel was tumbled at 30 r.p.m. for 20 ± 4 hr. The contents were allowed to settle for 30 min before withdrawing a 20 ml sample of the water phase for centrifugation. The salinity of the sample was adjusted to 2% (wt/wt) with sodium chloride before centrifugation. The sample was centrifuged (2500 r.p.m. for 10 min), and the supernatant was stored at 4°C prior to analysis. Leachate samples from the soil column studies did not require extraction.

2.4 Chemical and toxicological analyses of samples

2.4.1 Chemical analyses of extracts for PAH constituents

To quantify the occurrence of 14 PAH constituents in extracts of wastes and soil/waste mixtures, Method 8310 (USEPA 1982) was followed. Four millilitres of acetonitrile were added to triplicate aliquots of the extracts and concentrated over a 95–100°C water bath to evaporate the dichloromethane. The volume of the concentrate was adjusted to 1–5 ml with acetonitrile. A 3 µl aliquot of each concentrated sample was used for high performance liquid chromatographic (HPLC) analyses under the following chromatographic conditions: Wavelength 254 nm, isocratic for 1 min with a 40% acetonitrile/60% water eluent, followed by a linear gradient elution to 100% acetonitrile over 7 min, with a 3 min hold at 100% acetonitrile. A Perkin-Elmer Series 4 HPLC system equipped with UV detector (Perkin-Elmer Model LC-85B), integrator (Perkin-Elmer Model LC1-100), and a reverse phase column (Perkin-Elmer Model HC-ODS SIL-X) was used.

2.4.2 Analyses of extracts and leachates for mutagenic potential and Microtox™ toxicity

The Ames *Salmonella typhimurium* mammalian microsome assay was used to test the mutagenic potential of waste, soil, and soil/waste extracts initially and at the end of the

incubation study to observe mutagenic potential changes through time due to treatment by the soil medium. Prior to use in the mutagen assay, an aliquot of each concentrated extract was brought to dryness under a stream of purified air. Each residue was then diluted to a predetermined volume in dimethyl sulfoxide (DMSO) and sonicated for several minutes to ensure thorough mixing (Ma *et al.* 1983).

A frameshift mutagen indicator, *Salmonella typhimurium* tester strain TA-98 (provided by B. Ames, Department of Biology, University of California, Berkeley), was used with a rat liver homogenate (Aroclor 1254-induced S-9 fraction, Litton Bionetics, Inc. Kensington, MD). Based on the recommendation of Williams and Preston (1983), a preliminary test and a confirmatory test, involving duplicate plates, were performed for each acid or base/neutral extract waste sample following the plate incorporation procedures described by Maron and Ames (1983). For each test, five doses of the sample were evaluated with and without S9, one dose per plate, with duplicate plates per dose. Each confirmatory test involved repeating the assay using additional doses to provide the most active mutagenic response. One plate incorporation test, using triplicate plates, was used to determine the mutagenic potential of extracts obtained from the soil and soil/waste batch reactors. Controls used in each test included plates with no chemical addition, solvent controls with DMSO added, and positive controls with known mutagens added. The known mutagens, including daunomycin-HCL (a direct acting mutagen not requiring S-9 (mammalian microsome) activation to exhibit mutagenic potential) and 2-aminofluorene (an indirect acting mutagen requiring S-9 activation to exhibit mutagenic potential), were used as positive control mutagens in all tests. Toxicity of samples was evaluated by checking for the presence of background growth (lawn) on sample plates. A lawn that was sparse or absent on sample plates compared to control plates with no sample added indicated toxicity to the tester strain.

An automatic colony counter was used to count the number of revertants/plate. Results of the Ames assay were used to calculate a mutagenic ratio, i.e. the number of mutant colonies growing in the presence of a test sample compared to the number of spontaneous mutant colonies growing in the absence of a test sample. When spontaneous reversion rates (without test sample) were within the acceptable range proposed by DeSerres and Shelby (1979), a positive mutagenic response was defined as a mutagenic ratio (MR) greater than or equal to 2.5, where:

$$MR = \text{number of colonies with sample/number of colonies without sample.}$$

A test compound was considered to have a slight mutagenic response if the MR was less than 2.5 and greater than 2.0.

The Microtox™ assay was utilized to provide an indication of the acute toxicity of the WSF of the wastes, waste-amended soils from the incubation study, and of leachates generated in the column studies. Samples were analysed and EC50 values calculated according to the procedure in the Beckman Microtox™ System Operating Manual (Beckman 1982). If the EC50 of a sample, measured five minutes after sample addition at a temperature of 15°C (EC50 (5, 15°)) was greater than 100, the sample was considered non-toxic (NT).

3. Results and discussion

3.1 Apparent degradation of waste PAH constituents

Apparent degradation of total PAH constituents, on a mass basis, for each waste is summarized in Table 4. Changes in concentrations of each PAH compound are shown in Tables 5 and 6 for petroleum refining wastes and in Tables 7 and 8 for wood preserving wastes.

Generally, results indicated greater apparent degradation for lower molecular weight PAHs, which are non-carcinogenic, and less apparent degradation for higher molecular weight PAHs, which are carcinogenic or co-carcinogenic. The group of non-carcinogenic PAHs, including naphthalene, fluorene, phenanthrene, and anthracene, were compared with the group of carcinogenic and co-carcinogenic PAHs, including fluoranthene, pyrene, benzo(a)anthracene, and chrysene with regard to apparent degradation. Results are summarized in Table 9. Greater apparent degradation was indicated for the non-carcinogenic group of PAHs, ranging from 54–90% of mass added for the four wastes evaluated. The carcinogenic group of PAHs exhibited apparent degradation ranging from 24–53% of mass added for the four wastes. The greater apparent degradation of the non-carcinogenic PAHs was not unexpected since these compounds serve as carbon and energy sources for soil micro-organisms, while the carcinogenic PAHs generally cannot serve as ubiquitous carbon and energy sources for soil micro-organisms, but are believed to be degraded through co-oxidation processes (Park 1987).

Within the carcinogenic group of PAHs, reduction in concentration (Table 9) was directly correlated with oil and grease content of the waste (Table 1), i.e. decreases of 53, 41, 34, and 24% were associated with wastes of oil and grease contents of 52, 46, 37 and 35%, respectively. The presence of hydrocarbon substrates such as oil and grease can result in an overall increase in microbial activity and the co-oxidation of high molecular weight PAHs (Bossert *et al.* 1984, and Perry 1979). Co-oxidation was defined by Foster (1962*a*,*b*) as the condition where "non-growth" hydrocarbons are oxidized when present as co-substrates in a medium in which one or more different hydrocarbons are furnished for use for growth of the microbial biomass. Therefore, the extent of degradation of carcinogenic PAHs among the four wastes was correlated with the concentrations of oil and grease in the wastes, and may indicate the relative intensity of co-oxidation among the wastes.

These results agree with the findings of Keck (1989), who evaluated the apparent degradation of PAH compounds with and without an oily matrix. While the degradation of non-carcinogenic PAHs could not be correlated with the presence or absence of an oily matrix, carcinogenic and co-carcinogenic PAHs were found to disappear to a significantly greater extent from soils amended with complex wastes with an oily matrix. Results of the present study indicate that, in addition to the presence of an oily matrix, the concentration of oil and grease in a waste may be important in affecting the extent of co-oxidation of carcinogenic PAHs present in a waste/soil mixture.

3.2 Evaluation of detoxification of waste/soil mixtures

Results for mutagenicity using the Ames assay for the four waste/soil mixtures are presented in Fig. 1. Results are presented for each waste/soil mixture immediately after incorporation of waste into unacclimated Kidman sandy loam soil and after 354 days of incubation. No mutagenic potential was exhibited by API separator sludge and PCP-

TABLE 4

Apparent degradation of total PAH constituents* present in four complex wastes in unacclimated Kidman sandy loam soil

Complex waste	Initial waste concentration (mg kg^{-1} waste*)	Initial waste/soil concentration (mg kg^{-1} soil*)	Waste/soil concentration at day 354 (mg kg^{-1} soil*)	Decrease during incubation (%)
API separator sludge	14,999	2,489	1,768	29
Slop oil emulsion solids	51,120	6,646	3,802	43
Creosote sludge	293,700	2,769	1,524	45
PCP-creosote mixed sludge	259,100	490	141	71

* Results are presented for eight PAH compounds, which represent 96–98% of the mass of the 14 PAHs analysed in the complex waste samples.
† Results are presented on a dry weight of waste or soil basis.

TABLE 5
Apparent degradation of PAH constituents* in API separator sludge in unacclimated Kidman sandy loam soil

PAH constituent	Initial waste concentration (mg kg^{-1} waste†)	Initial waste/soil concentration (mg kg^{-1} soil†)	Waste/soil concentration at day 354 (mg kg^{-1} soil†)	Decrease during incubation (%)
Naphthalene	580 ± 87‡	68.9 ± 3.2‡	n.d.§	
Fluorene	29 ± 33	20.9 ± 3.3		
Phenanthrene	810 ± 140	150.0 ± 10.0	5.9 ± 1.7‡	100
Anthracene	110 ± 27	19.5 ± 2.5	69.0 ± 7.8	72
Fluoranthene	5,500 ± 290	857.0 ± 55.1	0.2 ± 0.0	54
Pyrene	6,000 ± 440	1,100.0 ± 100.0	763.0 ± 97.1	99
Benzo(a)anthracene	1,400 ± 58	100.0 ± 10.0	713.0 ± 104.0	11
Chrysene	570 ± 310	173.0 ± 11.6	150.0 ± 26.5	35
			66.5 ± 11.9	—
				62

* Results are presented for eight PAH compounds, which represent 96.2% of the mass of the 14 PAHs analysed in the waste sample.
† Results are presented on a dry weight of waste or soil basis.
‡ Results are expressed as mean concentration of three replicate analyses ± one standard deviation.
§ n.d. = Not detected.

TABLE 6

Apparent degradation of PAH constituents* in slop oil emulsion solids in unacclimated Kidman sandy loam soil

PAH constituent	Initial waste concentration (mg kg^{-1} waste†)	Initial waste/soil concentration (mg kg^{-1} soil†)	Waste/soil concentration at day 354 (mg kg^{-1} soil†)	Decrease during incubation (%)
Naphthalene	2,500 ± 700‡	143.0 ± 5.8‡	n.d.§	100
Fluorene	440 ± 300	53.0 ± 3.8	19.4 ± 2.3‡	63
Phenanthrene	3,600 ± 2,100	343.0 ± 11.6	196.0 ± 5.8	43
Anthracene	480 ± 93	55.0 ± 2.8	0.2 ± 0.0	100
Fluoranthene	18,000 ± 5,000	2,600‖	1,600.0 ± 173.0	38
Pyrene	23,000 ± 6,700	3,000‖	1,700.0 ± 173.0	43
Benzo(a)anthracene	2,000 ± 1,100	320‖	233.0 ± 25.0	27
Chrysene	1,100 ± 150	132¶	53.4 ± 7.7	60

* Results are presented for eight PAH compounds, which represent 98% of the mass of the 14 PAHs analysed in the waste sample.
† Results are presented on a dry weight of waste or soil basis.
‡ Results are expressed as mean concentration of three replicate analyses ± one standard deviation.
§ n.d. = not detected.
‖ One sample was analysed.
¶ Initial concentration was calculated from loading rate and waste concentration.

TABLE 7

Apparent degradation of PAH constituents* in creosote sludge in unacclimated Kidman sandy loam soil

PAH constituent	Initial waste concentration (mg kg^{-1} waste†)	Initial waste/soil concentration (mg kg^{-1} soil†)	Waste/soil concentration at day 354 (mg kg^{-1} soil†)	Decrease during incubation (%)
Naphthalene	28,000 ± 1,200‡	273.0 ± 11.5‡	n.d.§	100
Fluorene	23,000 ± 5,900	177.0 ± 11.5	72.1 ± 18.9‡	59
Phenanthrene	76,000 ± 15,00	833.0 ± 23.0	500.0 ± 98.5	40
Anthracene	15,000 ± 6,800	243.0 ± 110.0	131.0 ± 52.0	46
Fluoranthene	72,000 ± 17,00	567.0 ± 11.5	397.0 ± 90.7	30
Pyrene	64,000 ± 12,00	573.0 ± 5.8	353.0 ± 76.4	38
Benzo(a)anthracene	7,400 ± 1,600	52.5 ± 0.7	36.0 ± 8.0	31
Chrysene	8,300 ± 150	50.5 ± 1.0	35.1 ± 8.0	30

* Results are presented for eight PAH compounds, which represent 96% of the mass of the 14 PAHs analysed in the waste sample.
† Results are presented on a dry weight of waste or soil basis.
‡ Results are expressed as mean concentration of three replicate analyses ± one standard deviation.
§ n.d. = not detected.

TABLE 8

Apparent degradation of PAH constituents* in PCP-creosote mixed sludge in unacclimated Kidman sandy loam soil

PAH constituent	Initial waste concentration (mg kg^{-1} waste†)	Initial waste/soil concentration (mg kg^{-1} soil†)	Waste/soil concentration at day 354 (mg kg^{-1} soil†)	Decrease during incubation (%)
Naphthalene	42,000 ± 28,000‡	n.d.§	n.d.	—
Fluorene	22,000‖	49.2 ± 1.7‡	1.6 ± 1.8‡	97
Phenanthrene	52,000 ± 6,200	120.0 ± 0.0	4.2 ± 0.8	97
Anthracene	11,000 ± 6,800	69.4 ± 7.2	17.5 ± 5.4	75
Fluoranthene	46,000 ± 6,200	73.3 ± 63.5	55.3 ± 14.7	25
Pyrene	56,000 ± 13,000	143.0 ± 5.8	37.5 ± 28.9	74
Benzo(a)anthracene	16,000 ± 2,400	14.0 ± 1.6	10.9 ± 1.5	22
Chrysene	6,900 ± 2,200	20.7 ± 1.8	14.2 ± 1.7	31

* Results are presented for 8 PAH compounds, which represent 96% of the mass of the 14 PAHs analysed in the waste sample.
† Results are presented on a dry weight of waste or soil basis.
‡ Results are expressed as a mean concentration of three replicate analyses ± one standard deviation.
§ n.d. = not detected.
‖ One sample was analysed.

TABLE 9

Apparent degradation of four non-carcinogenic and four carcinogenic PAH compounds in four complex wastes in unacclimated Kidman sandy loam soil during 354 days incubation

PAH Group	Complex waste/soil mixture			
	API separator sludge	Slop oil emulsion solids	Creosote sludge	PCP-creosote mixed sludge
Non-carcinogenic				
Initial concentration (mg kg^{-1})	259 ± 18*	595 ± 19	1,527 ± 150	239 ± 9
Final concentration (mg kg^{-1})	75 ± 9	216 ± 8	703 ± 154	23 ± 8
Mass removed (%)	71	64	54	90
Carcinogenic				
Initial concentration (mg kg^{-1})	2,230 ± 171†	6,052‡	1,243 ± 19	251 ± 64
Final concentration (mg kg^{-1})	1,693 ± 236	3,587 ± 373	821 ± 183	118 ± 47
Mass removed (%)	24	41	34	53

* Results are expressed as mean concentration (mg kg^{-1}) of three replicates ± one standard deviation for the total of four non-carcinogenic PAHs, including naphthalene, fluorene, phenanthrene and anthracene.

† Results are expressed as mean concentration (mg kg^{-1}) of three replicates ± one standard deviation for the total of four carcinogenic and co-carcinogenic PAHs, including fluoranthene, pyrene, benzo(a)anthracene and chrysene.

‡ Results are based on analysis of one sample for three compounds and a calculated initial concentration for one compound.

creosote waste/soil mixtures either initially or after 400 days of incubation, indicated by the observation that all values of mutagenic ratio are two or less. Slop oil emulsion solids and creosote sludge/soil mixtures exhibited a positive mutagenic potential after initial incorporation of waste into soil. The dose-response curve for creosote sludge/soil mixture shows a decrease in the value of the mutagenic ratio as the dose increases beyond 100 mg soil/plate in the presence of S9 (enzyme activation), indicating that the waste/soil mixture was toxic to the Ames bacterium used (*Salmonella typhimurium* strain TA-98) at these concentrations. Results for slop oil emulsion solids indicated that a positive mutagenic potential was obtained both with and without the presence of mammalian activated enzymes. After approximately 350 days of incubation in soil, the waste/soil mixtures containing these wastes did not exhibit a positive mutagenic response. Therefore, detoxification of these wastes was demonstrated through reduction of mutagenic potential.

The decrease in mutagenic potential of slop oil emulsion solids- and creosote sludge-amended soil samples waste/soil extracts (Fig. 1) correlated with the apparent degradation and final concentrations of carcinogenic PAH compounds (Table 9). No mutagenic activity ($MR = 1.0$) was present in organic extracts of the Kidman sandy loam soil before waste application.

Microtox™ toxicity values for the four wastes evaluated are summarized in Table 10. The toxicities of WSFs of three waste/soil mixtures, creosote sludge, PCP-creosote mixed sludge, and slop oil emulsion solids, exhibited only small decreases, as measured by the Microtox™ assay, during the incubation study (Fig. 2). An increase in the toxicity of the WSF of the API separator sludge-amended soil was observed during the incubation period (Fig. 2). At Day 0, EC50 was 82.1 ± 19.35%, while by Day 340, toxicity had increased to 18.3 ± 3.2%. Control soil samples that were not amended with wastes did not exhibit toxicity (EC ≥ 100%) during the course of the experiment.

Similar results were obtained by Symons and Sims (1988) for a composite petroleum

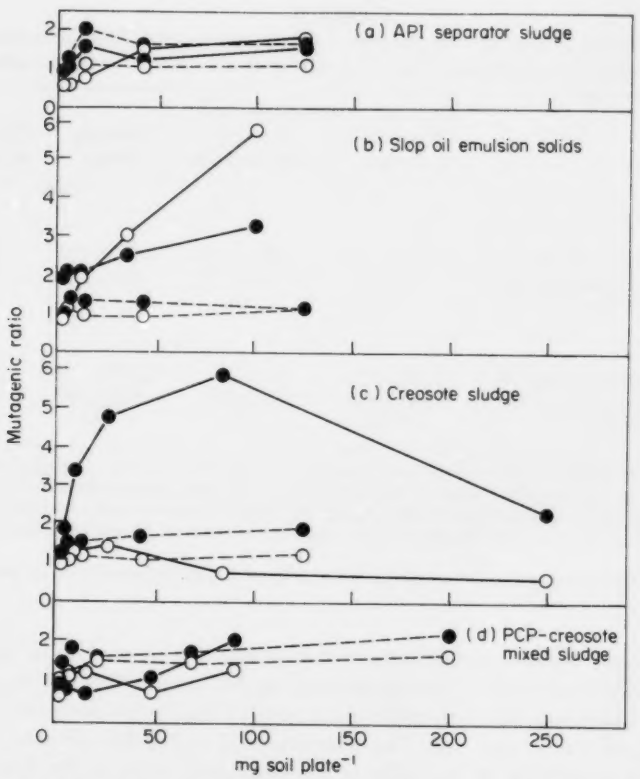


Fig. 1. Response of TA-98 when exposed to dichloromethane extracts of a Kidman sandy loam soil when initially amended with four complex wastes (——) and after 354 days incubation (– – –) in the presence of S-9 (●) and in its absence (○). The mean of three determinations/dilution is presented.

TABLE 10

WSF toxicity of complex wastes, as measured by the Microtox™ assay

Complex waste	EC50 (5,15')*(vol %)†
API separator sludge	0.3
Slop oil emulsion solids	0.7
Creosote sludge	6.0
PCP-creosote mixed sludge	1.3

* EC50 (5,15') denotes the conditions of the test, i.e. reading light output five minutes after sample addition at a temperature of 15°C.
† Results given are means of two replicates.

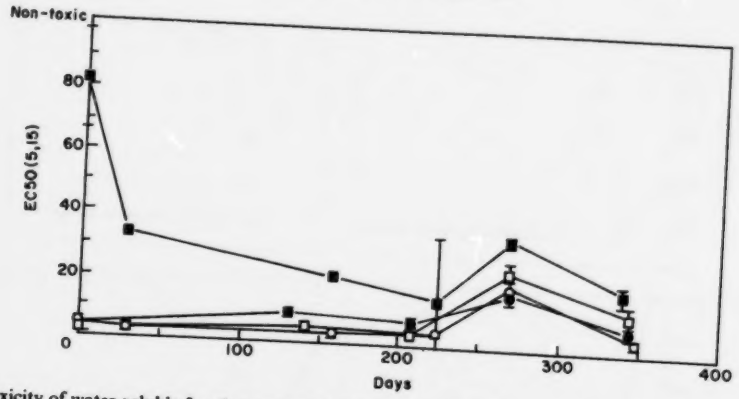


Fig. 2. Toxicity of water soluble fraction measured with the Microtox™ assay with incubation time for PCP-creosote mixed sludge (—□—), creosote sludge (—○—), API separator sludge (—■—), and slop oil emulsion solids (—●—) mixed with a Kidman sandy loam soil. EC50 (5, 15') denotes the effective concentration (vol/vol) of water soluble extract that reduces light emission of the Microtox™ organisms by 50% five minutes after exposure to the test solution at 15°C. Values presented prior to 250 days of incubation are the average and standard deviation of duplicate samples. Values presented after 250 days were determined from single samples with a 95% confidence interval.

waste comprised of equal proportions of API separator sludge, slop oil emulsion solids and dissolved air flotation float incubated in the Kidman sandy loam soil used in the experiments described in this study. After incubation of the composite waste at 4% oil and grease in the soil (waste wet weight/soil dry weight), which was approximately the same loading used in the experiments reported for this study for the two petroleum wastes (Table 3), WSF toxicity decreased from an EC50 value of 6.5% immediately after waste incorporation to a value of 27.8% after 180 days of incubation. This slight decrease in toxicity was observed even though substantial degradation of PAH compounds, both carcinogenic and non-carcinogenic, was measured, which is similar to results obtained for this study.

Results for toxicity of leachate samples generated in the laboratory column studies are presented in Fig. 3. Immediately after waste incorporation into soil, Microtox™ toxicity was observed for a mimimum of 10 column volumes for the wood preserving wastes; less leachate toxicity was exhibited by the petroleum wastes. After approximately one year of incubation in Kidman sandy loam soil, leachate toxicity was still present for the wood preserving waste/soil mixtures and for the petroleum refining waste/soil mixtures for the first several column volumes of leachate generated. Leachates from columns containing soil only did not exhibit any degree of toxicity (EC50 $\geq$ 100%).

For the composite petroleum waste evaluated by Symons and Sims (1988), some Microtox™ toxicity was still observed in continuously fed unsaturated laboratory columns after 180 days incubation in Kidman sandy loam soil (EC50 value of 84.4%).

Degradation of PAH compounds is known to result in the production of more polar intermediate metabolites (Park 1987, Park *et al.* 1988), which are also more water soluble and more likely to leach from the soil solid phase. Therefore, toxicity of the WSF and leachates in a soil system impacted by PAH-containing wastes is anticipated and was observed in this study. Also, contamination of unacclimated soil at concentrations that are highly toxic, as determined by the Microtox™ assay, did not decrease to background levels after an incubation of approximately one year.

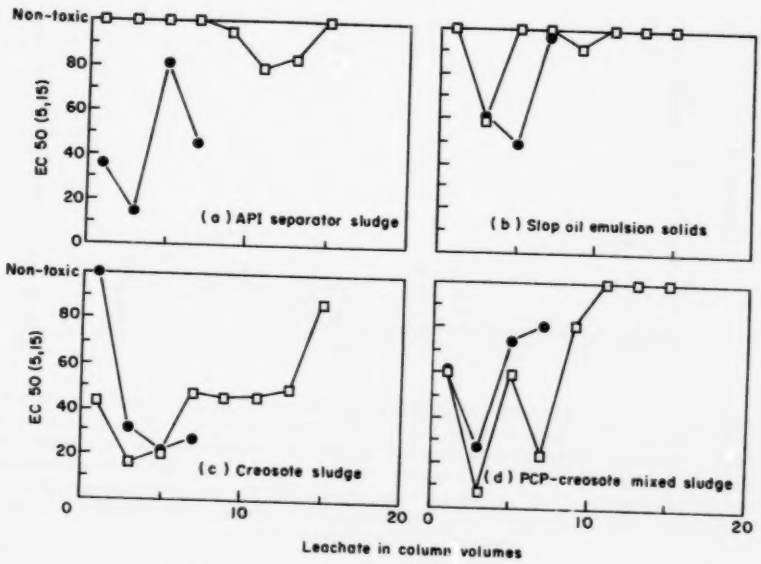


Fig. 3. Toxicity of leachates from complex wastes in a Kidman sandy loam soil immediately after waste loading (—□—) and after 350 days of incubation (—●—). EC50 (5, 15') denotes the effective concentration (vol/vol) of water soluble extract that reduces light emission of the Microtox™ organisms by 50% five minutes after exposure to the test solution at 15°C. Day 0 values represent the average of duplicate samples. Final values after incubation are single determinations.

5. Conclusions

In this study, the extent of apparent degradation of four carcinogenic PAHs in wood preserving and petroleum refining wastes in unacclimated soil was directly correlated with the concentration of oil and grease in each waste, and may have been due to the relative stimulation of co-oxidation by the oil and grease in the waste/soil samples. The extent of apparent degradation of four non-carcinogenic PAHs was greater than the degradation of carcinogenic PAHs for all wastes, and was not correlated with oil and grease content of the wastes.

Toxic concentrations of the wastes, as characterized by the Microtox™ assay, resulted in the production of water soluble fraction and leachate toxicity one year after application of the waste to the soil, presumably due to the formation of more water soluble metabolites. These results were observed even though no mutagenicity of the soil solid phase was observed after one year incubation in the soil. Therefore, the Microtox™ bioassay provided a method for assessing the long term treatment performance and the relative extent of detoxification of the four complex wastes in the unacclimated Kidman sandy loam soil.

This study also demonstrated that an integration of information concerning apparent degradation of hazardous constituents of complex wastes with bioassay information represents an approach for evaluation of the effectiveness of in-situ bioremediation of contaminated soil/waste systems. When combined with information from waste, site and soil characterization studies, the data generated in treatability studies may be used in predictive mathematical models to: (1) evaluate the effectiveness of use of on-site bioremediation for treatment of a specific waste in a specific soil system; (2) develop

appropriate containment structures to prevent unacceptable waste transport from the treatment zone; and (3) design performance monitoring strategies.

Although this research was conducted for a period of approximately one year, shorter term treatability studies using the approach described in this study may be used to investigate methods to enhance degradation and detoxification of waste-contaminated soils in order to achieve timely and efficient bioremediation. Factors that may be evaluated in laboratory studies to enhance degradation and detoxification include: (1) soil incorporation or mixing to reduce the initial (toxic) concentration; (2) application of wastes more frequently and at lower concentrations to acclimate soils to toxic complex wastes and to avoid application of toxic concentrations; (3) addition of mineral nutrients; (4) addition of microbial carbon and energy sources to stimulate co-oxidation; and (5) use of different soil types as the treatment medium.

6. Acknowledgments

Support for this research project was provided by the Robert S. Kerr Environmental Research Laboratory, USEPA, Ada, OK under Co-operative Agreement Nos. 810979 and 813211. Although this research was funded by the USEPA, this article has not been subject to USEPA review and therefore does not necessarily reflect the views of the Agency. The authors would like to thank Joan McLean, Lauren Hastings, Michael Walsh, Robert Bramblett, Dr William Doucette, and Dr Darwin Sorensen of the professional and technical staff of the Utah Water Research Laboratory, Utah State University, for conducting the laboratory analyses for this study.

References

Ames, B. N., McCann, J. & Yamasaki, E. (1975), Methods for detecting carcinogens and mutagens with the *Salmonella* mammalian-microsome mutagenicity test, *Mutation Research, 31*, 347–364.

AWMA/EPA (1989), *Proceedings of the International Symposium on Hazardous Waste Treatment: Biosystems for Pollution Control*. Air and Waste Management Association and the USEPA, Cincinnati, Ohio, February 20–23.

Beckman Instruments (1982), *Beckman Microtox™ Systems Operating Manual*, Microbial Operations, Carlsbad, CA.

Bos, R. P., Hulshof, C. T. J., Theuws, J. L. G. & Henderson, P. T. (1983), Mutagenicity of creosote in the *Salmonella* microsome assay, *Mutation Research, 119*, 21–25.

Bos, R. P., Theuws, J. L. G., Leijdekkers, Ch.-M. & Henderson, P. T. (1984), The presence of the mutagenic polycyclic aromatic hydrocarbons benzo(a)pyrene and benz(a)anthracene in creosote P1, *Mutation Research, 130*, 153–158.

Bossert, I., Kachel, W. M. & Bartha, R. (1984), Fate of hydrocarbons during oily sludge disposal in soil, *Applied and Environmental Microbiology, 47*, 763–767.

Cerniglia, C. E., Althaus, J. A., Evans, F. E., Freeman, J. P., Mitchum, R. K. & Yang, S. K. (1983), Sterochemistry and evidence for an arene oxide-NIH shift pathway in the fungal metabolism of naphthalene, *Chemical and Biological Interactions, 44*, 119–132.

Cerniglia, C. E. & Yang, S. K. (1984), Steroselective metabolism of anthracene and phenanthrene by the fungus *Cunninghamella elegans*, *Applied and Environmental Microbiology, 47*, 119–124.

Coover, M. P. & Sims, R. C. (1987), The rate of benzo(a)pyrene apparent loss in a natural and manure amended clay loam soil, *Hazardous Wastes & Hazardous Materials 4*, 151–158.

Coover, M. P., Sims, R. C. & Doucette, W. J. (1987), Extraction of polycyclic aromatic hydrocarbons from spiked soil, *Journal of the Association of Official Analytical Chemists, 70*, 1018–1020.

Cripps, R. E. & Watkinson, R. J. (1978), Polycyclic aromatic hydrocarbons: Metabolism and environmental aspects, In *Developments in Biodegradation of Hydrocarbons* (Watkinson, R. J., ed.), Applied Science Publishers Ltd., Essex, England, pp 113–134.

Crosby, D. G. (1981), Environmental Chemistry of pentachlorophenol, *Pure and Applied Chemistry, 53*, 1052–1080.

DeSerres, F. J. & Shelby, M. D. (1979), Recommendations on data production and analysis using the *Salmonella* microsomal mutagenicity assay. *Mutation Research, 64*, 159–165.

Donnelly, K. C., Brown, K. W., Thomas, J. C. & Davol, P. (1985), Evaluation of the hazardous characteristics of two petroleum wastes, *Hazardous Wastes & Hazardous Materials, 2*, 191–207.

Donnelly, K. C., Davol, P., Brown, K. W., Estiri, M. & Thomas, J. C. (1986), Mutagenic activity of two soils amended with a wood-preserving waste, *Environmental Science and Technology, 21*, 57–72.

Engineering Foundation, (1988), *Proceedings, Conference on Biotechnology Applications in Hazardous Waste Treatment*, Engineering Foundation Conferences, Longboat Key, Florida, October 31–November 4.

Foster, J. W. (1962*a*), Hydrocarbons as substrates for micro–organisms. *Antonie van Leeuwenhoek Journal of Microbiology and Serology, 28*, 241–274.

Foster, J. W. (1962*b*), Bacterial oxidation of hydrocarbons, In *Oxygenases* (Hayaishi O., ed.) Academic Press Inc., New York.

Gibson, D. T., Mahadevan, V., Jerina, D. M., Yagi, H. & Yeh, H. J. C. (1975), Oxidation of the carcinogens benzo(a)pyrene and benz(a)anthracene to dihydrodiols by a bacterium, *Science 189*, 295–297.

Hazardous Materials Control Research Institute (HMCRI) (1988), *Use of Genetically Altered or Adapted Organisms in the Treatment of Hazardous Wastes*, Specialty Conference, Washington, D.C., November 30–December 2.

Herbes, S. E. & Schwall, L. R. (1978), Microbial transformation of PAHs in pristine and petroleum contaminated sediments, *Applied and Environmental Microbiology, 35*, 306–316.

Keck, J. (1989), *Co-oxidation of Polynuclear Aromatic Hydrocarbons in Soil Systems*, MS Thesis, Department of Civil and Environmental Engineering, Utah State University, Logan, Utah.

Ma, C. Y., Ho, C.-h., Quincy, R. B., Guerin, M. R., Rao, T. K., Allen, B. E. & Epler, J. L. (1983), Preparation of oils for bacterial mutagenicity testing, *Mutation Research, 118*, 15–24.

Maron, D. M. & Ames, B. N. (1983), Revised methods for the *Salmonella* mutagenicity test, *Mutation Research, 113*, 173–215.

Matthews, J. E. & Bulich, A. A. (1984), A toxicity reduction test system to assist in predicting land treatability of hazardous wastes, In *Hazardous and Industrial Solid Waste Testing: Fourth Symposium*. (Petros, J. K. Jr., Lacy, W. J. & Conway, R. A., eds.) STP-886, American Society of Testing and Materials, Philadelphia, PA, 176–191.

Matthews, J. E. & Hastings, L. (1987), Evaluation of toxicity test procedure for screening treatability potential of waste in soil, *Toxicity Assessment: An International Quarterly, 2*, 265–281.

McCann, J. R., Choi, R., Yamasaki, E., & Ames, B. N. (1975), Detection of carcinogens as mutagens in the *Salmonella* microsome test: Assay of 300 chemicals, *Proceedings of the National Academy of Science, 72*, 5135–5139.

McGinnis, G. D., Borazjani, H., McFarland, L. K., Pope, D. F., & Strobel, D. A. (1988), *Characterization and Laboratory Soil Treatability Studies for Creosote and Pentachlorophenol Sludges and Contaminated Soil*. EPA/600/2- 88/055. USEPA, Ada, OK.

McKenna, E. J. (1977), *Biodegradation of Polynuclear Aromatic Hydrocarbon Pollutants: By Soil and Water Micro-organisms*, American Institute of Chemical Engineers, 70th Annual Meeting, Nov. 13–17, New York, NY.

Omenn, G. S., ed. (1987), *Environmental Biotechnology–Reducing Risks from Environmental Chemicals through Biotechnology*, Proceedings, Conference, University of Washington, Seattle, Washington, July 19–23, Plenum Press, New York.

Park, K. S. (1987), *Degradation and Transformation of Polycyclic Aromatic Hydrocarbons in Soil Systems*, Ph.D. Dissertation, Department of Civil and Environmental Engineering, Utah State University, Utah.

Park, K. S., Sims, R. C., Doucette, W. J. & Matthews, J. E. (1988), Biological transformation and detoxification of 7,12-dimethylbenzanthracene in soil systems, *Journal Water Pollution Control Federation, 60*, 1822–1825.

Perry, J. J. (1979), Microbial co-oxidations involving hydrocarbons, *Microbiological Reviews, 43*, 59–72.

Sims, R. C., Sims, J. L. & Dupont, R. R. (1984), Human health effects assays, *Journal Water Pollution Control Federation, 56*, 791–800.
Sims, R. C., Sims, J. L. & Dupont, R. R. (1985), Human health effects assays, *Journal Water Pollution Control Federation, 57*, 728–742.
Sims, R. C., Sims, J. L. & Dupont, R. R. (1986), Human health effects assays, *Journal Water Pollution Control Federation, 58*, 703–717.
Sims, R. C., Sims, J. L. & Dupont, R. R. (1987), Human health effects assays, *Journal Water Pollution Control Federation, 59*, 601–614.
Sims, R. C., Sims, J. L. & Dupont, R. R. (1988), Human health effects assays, *Journal Water Pollution Control Federation, 60*, 1093–1196.
Symons, B. D. & Sims, R. C. (1988), Assessing detoxification of a complex hazardous waste, using the Microtox™ bioassay, *Archives of Environmental Contamination and Toxicology, 17*, 497–505.
Sims, R. C. (1989), Overview of bioremediation in soil and groundwater—theoretical and practical considerations, In *Proceedings, Symposium on Bioremediation of Wood Treating Wastes* (McGinnis, G. ed.), Mississippi State University, Mississippi State, Mississippi, March 14–15.
USEPA (1974), *Development Document for Effluent Limitation Guidelines and New Source Performance Standards for the Plywood. Hardboard and Wood Preserving Segment of the mber Products Processing Point Source Category*. EPA-440/1-74-023a. USEPA, Washington, DC.
USEPA (1982), *Test Methods for Evaluating Solid Waste, Physical-chemical Methods*, 2nd ed. SW-846. USEPA, Washington, DC.
USEPA (1984), *Creosote-Special Review Position Document 213*. USEPA, Washington, DC.
USEPA (1986*a*), *Ambient Aquatic Life Water Quality Criteria for Pentachlorophenol*. USEPA, Duluth, MN.
USEPA (1986*b*), *Permit Guidance Manual on Hazardous Waste Land Treatment Demonstrations*. EPA-530/SW-86-032. USEPA, Washington, DC.
USEPA (1986*c*), *Waste-Soil Treatability Studies for Four Complex Wastes: Methodologies and Results*. Vol. 1: *Literature Assessment, Waste-Soil Characterization, Loading Rate Selection*; and Vol. 2: *Waste Loading Impacts on Soil Degradation, Transformation, and Immobilization*. EPA/600/6-86/003a and b, USEPA, Ada, OK.
USEPA (1989), *Proceedings, Workshop on Bioremediation of Hazardous Waste Sites*. USEPA, Philadelphia, PA.
Williams, L. R. & Preston, J. R. (1983), *Interim Procedures for Conducting the Salmonella/ Microsomal Mutagenicity Assay (Ames Test)*. USEPA, Las Vegas, NV.